Remarks

Claims 1-20 and 46-129 are cancelled without prejudice or disclaimer. Applicant reserves the right to pursue the subject matter of the cancelled claims in continuing applications.

Claims 21, 26, 29, 31 and 42 are amended. Support for amendment to claim 21 can be found on page 2 lines 4-8, and page 44 lines 12-18. Claims 26 and 29 are amended to correct typographical errors. Claims 31 and 42 are amended to recite proper antecedent basis.

New claims 130 and 131 are added. Support for these new claims can be found on page 31 lines 19-21.

Claims 21-45, 130 and 131 are pending, with claims 33, 37 and 39 being withdrawn based on a species election. Upon allowability of the generic claim (claim 21), examination of these withdrawn claims is requested.

No new matter has been added.

Rejections under 35 U.S.C. §112

Claims 21-32, 34-36, 38 and 40-45 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 21 is rejected, according to the Examiner, for not being clear regarding "whether contacting a biological sample with the said polypeptide is designed to have an inhibitory effect or a non-inhibitory effect". Applicant respectfully disagrees. The claim embraces both situations. The sample may be analyzed for the presence of compounds or activities that covalently modify the histone polypeptide and thereby increase or decrease the level of binding between the modified histone polypeptide and the histone-modification-specific binding domain.

Claim 21 is further rejected because, according to the Examiner, "the step of monitoring the level of FRET in the biological sample is not an actual step ... (since) ... there is no stated end result to this step nor is it involved in a cause-effect process step". Claim 21 is now amended to recite a cause-effect process step that relates histone covalent modification with FRET level. Support for this amendment can be found, for example, in the specification on page 2, lines 4-8.

Claim 32 is rejected because, according to the Examiner, "FHA and WW are made up abbreviations for polypeptide domains" and therefore are said to not distinctly claim the subject matter.

Applicant respectfully disagrees. A person of skill in the art will recognize the names of these domains and be aware of their properties. For example, page 15 lines 7-8 of the specification cites Yaffe *et al.* (Yaffe, M. B. & Elia, A. E. *Curr. Opin. Cell Biol.* 13, 131-138 (2001)), a publication which describes both FHA and WW domains. FHA and WW domains are known to be present in a variety of proteins. For example, a PubMed search for the term "WW domain" results in over 200 research articles, including at least 15 review articles, that refer to WW domains. Similarly, a PubMed search for "FHA domain" results in over 50 research articles, including 8 review articles, that refer to FHA domains. Accordingly, the terms FHA and WW are not "made up terms" but rather are terms of definite meaning to those of ordinary skill in the art.

Claim 34 is rejected because, according to the Examiner, "GCN5, P/CAF, $TAF_{II}250$, CBP, BRG1, Swi2, and Sth1 are made up abbreviations for peptides and polypeptide domains" and therefore are said to not distinctly claim the subject matter.

Applicant respectfully disagrees. A person of skill in the art will recognize the names of these domains and be aware of their properties. On page 15, lines 17-18 the specification cites several references that describe the aforementioned peptide and polypeptide domains, including *FEBS Lett* 513(1):124-8 (2002), *Front Biosci* 6:D1019-23 (2001); and *Nat Struct Biol* 6(7):601-4 (1999). Moreover, the Examiner has cited and relied on art that refers to GCN5, SWI, $TAF_{II}250$, BRG1 and P/CAF domains. (See, for example, Agalioti et al.) Further support that the art is familiar with these terms can be found through PubMed searches that turn up numerous references referring to each term. Accordingly, these terms are not "made up terms" but rather are terms of definite meaning to those of ordinary skill in the art.

Claim 35 is rejected because, according to the Examiner, "HP1, MRG15, MRG-1, cynCDY, Hrp3, dMi-2, CHD5, Swi6, and pdd3p are made up abbreviations for peptides and polypeptide domains" and therefore are said to not distinctly claim the subject matter.

Applicant respectfully disagrees. On page 15, lines 19-20 the specification cites *Nature* 407(6802):405-9 (2000), which describes the aforementioned peptide and polypeptide domains. These domains are known in the art and are present in a variety of proteins. Further support that the art is familiar with these terms can be found through PubMed searches that turn up numerous references referring to each term. Accordingly, these terms are not "made up terms" but rather are terms of definite meaning to those of ordinary skill in the art.

In view of the foregoing, terms recited in claims 32, 34 and 35 and thus the claims themselves are considered definite. Reconsideration and withdrawal of the rejection under 35 U.S.C. §112, second paragraph is respectfully requested.

Rejections under 35 U.S.C. §103

WO 01/33199 in view of Akhtar et al.

Claims 21-32, 34-36, and 40-45 are rejected under 35 U.S.C. §103(a) as being unpatentable over WO 01/33199 in view of Akhtar et al. Claim 21 is amended to recite "covalent" histone modification. Applicant traverses in part.

The claims, as now amended, relate to monitoring the level of histone *covalent* modification in a sample by contacting the sample with a fusion reporter protein with a core comprising a histone-modification-specific binding domain conjugated to a histone polypeptide, where the core is flanked by donor and acceptor fluorophores. Covalent modification of the histone polypeptide after contact with the sample impacts binding of the histone-modification-specific binding domain to the modified histone polypeptide. For example, the covalent modification may be an acetylation or a deacetylation event, resulting in increased or decreased FRET levels respectively. As another example, the covalent modification may be a methylation or a demethylation event, resulting in increased or decreased FRET levels respectively. The histone polypeptide of the reporter protein may be, for example, acetylated or methylated prior to contact with the biological sample, in which case deacetylation or demethylation activities in the biological sample are being assayed in the claimed method.

WO 01/33199 teaches probes that are fusion proteins consisting of two fluorophores attached by a linker and bracketed by (a) a mimic peptide that mimics a target substance and (b) a target binding site peptide that can bind either the mimic peptide or the target substance. The

probes of WO 01/33199 assay for substances that *bind* to the target binding site peptide and thereby inhibit or stimulate binding of that domain to the mimic peptide as indicated by FRET. WO 01/33199 states that the probes "could be to any substance ... including ... peptides such as ... kinases; post-translational modification sites, for example, phosphorylation, glycosylation or farnsylation sites." It does not however contemplate monitoring covalent modification of the probe itself. It also does not teach a probe that is specific for acetylation events.

Akhtar et al. is a research paper relating to gene dosage compensation of single male X chromosomes in Drosophila. It focuses primarily on the binding interactions of the histone acetyltransferase MOF with RNA via its chromodomain. The paper states that "chromodomains have so far not been shown to contact proteins or peptides". (See page 408, first column, last paragraph.) Binding interactions are detected using gel electrophoretic methods.

A prima facie case of obviousness requires a motivation or suggestion to modify or combine references, a reasonable expectation of success relating to such modification or combination, and the modification or combination must provide each and every limitation of the pending claims.

A prima facie case for obviousness has not been made. There is no motivation to combine WO 01/33199 with Akhtar et al. because WO 01/33199 relates to screening methods for modulators of binding interactions and Akhtar et al. is a research paper studying the mechanism of gene dosage compensation. The motivation is so lacking that it is not even clear based on the teachings of these references how one of ordinary skill in the art would combine these references, or what the a combination would yield. For at least these same reasons, there also can be no reasonable expectation of success.

Even if the combination was proper (and Applicant maintains it is not), it would not provide each and every limitation of the pending claims. WO 01/33199 does not teach a method that monitors *covalent* modification of *histones*. It does not teach a probe designed to undergo covalent modification at one domain (e.g., the histone polypeptide). It does not teach the particular claimed histone polypeptide sequences. It further does not teach a probe having a core flanked by the FRET acceptor and donor. Akhtar et al. does not provide the deficiencies of WO 01/33199. At best, the combination might result in use of a probe of WO01/33199 for assaying modulators of the binding between MOF and roX2 RNA. This is not the claimed invention.

The Examiner does not provide the rationale for combining the references. The Examiner states that "it would have been obvious ... to use a H4 histone polypeptide in a fusion protein reporter construct", citing page 408 of Akhtar et al. and page 22 of WO 0133199. Page 408 of Akhtar et al. refers to the specificity of binding of MOF chromodomain to RNA, and states that *chromodomains were not known at that time to contact protein or peptides*. Page 22 of WO 01/33199 states that the probes "could be to any substance ... including ... peptides such as ... kinases; post-translational modification sites, for example, phosphorylation, glycosylation or farnsylation sites." Respectfully, these teachings do not yield the requisite motivation.

Reconsideration and withdrawal of the rejection under 35 U.S.C. §103(a) in view of WO 01/33199 and Akhtar et al. is respectfully requested.

WO 01/33199 in view of Agalioti et al.

Claims 21, 26-28, 31-32, 35-36 and 41 are rejected under 35 U.S.C. §103(a) as being unpatentable over WO 01/33199 in view of Agalioti et al.

The teachings and deficiencies of WO 01/33199 have been discussed above. Agalioti et al. is a research paper that proposes a theory of a transcriptional histone acetylation code. The reference reports histone H3 and H4 acetylation by GCN5 acetyltransferase in the area of the IFN-β gene enhancer. The reference further reports that the location of lysine acetylation on H4 controls recruitment of specific factors such as SWI/SNF and TFIID, thereby providing a mechanism of histone-mediated IFN-β transcriptional activation. Acetylation and recruitment are detected using gel electrophoresis methods. Agalioti et al. reports the use of acetylated histone N termini peptides or bromodomain polypeptides to compete away the interaction between acetyl-lysine histone N termini and bromodomains.

A prima facie case of obviousness has not been made. There is no motivation to combine the teachings of WO 01/33199 with those of Agalioti et al. WO 01/33199 teaches a method for identifying inhibitors or stimulators of binding interactions using FRET as a readout. It does not contemplate use of its assay for detecting covalent modification of its probes and there is no reasonable basis for expanding its meaning to embrace the covalent modification assay presently claimed. Agalioti et al. teaches specific histone acetylation and the downstream effects of such acetylation using gel electrophoretic methods. It not clear based on the teachings of these references how one of ordinary skill in the art would combine these references, or what

the combination would yield. For at least these same reasons, there also can be no reasonable expectation of success.

Even if the combination was proper (and Applicant maintains it is not), it does not provide each and every limitation of the pending claims. The deficiencies of WO 01/33199, as relating to the claimed invention, are described above. Agalioti et al. does not cure these deficiencies, and thus the combination cannot yield the claimed invention. At a minimum, Agalioti et al. does not teach the reporter proteins of the pending claims. In addition and as stated above, the result of any possible combination is unclear. For example, one could arrive at a method for detecting binding interactions (as in WO 01/33199) using an electrophoretic method (as in Agalioti et al.). This is not the claimed invention.

Reconsideration and withdrawal of the rejection under 35 U.S.C. §103(a) in view of WO 01/33199 and Agalioti et al. is respectfully requested.

WO 01/33199 in view of Reichheld et al.

Claims 21, 26-28, 31-32, 35-36 and 41 are rejected under 35 U.S.C. §103(a) as being unpatentable over WO 01/33199 in view of Reichheld et al.

The teachings of WO 01/33199 have been discussed above. Reichheld et al. is a research paper analyzing regulation of histone gene expression during the cell cycle in plants. Reichheld et al. examines the interactions of transcription factors with DNA at the *promoter* of histones H3 and H4. Reichheld et al. suggests that post-translational modification of *transcription factors* plays a part in regulating expression of histones H3 and H4. Contrary to the Examiner's position, Reichheld et al. does not teach the *amino acid* sequence of SEQ ID NO:18. The Examiner refers to Fig. 4 as teaching this sequence, however Fig. 4 provides a *nucleotide* sequence corresponding to a histone promoter sequence. Importantly, the sequence is provided in order to demonstrate transcription factor binding to the promoter. The reference says nothing about covalent modification of histones.

A prima facie case of obviousness has not been made. There is no motivation to combine the teachings of WO 01/33199 with those of Reichheld et al. at least because WO 01/33199 reports a probe for modulators of binding interactions using FRET while Reichheld et al. reports binding of transcription factors to DNA using electrophoretic methods. However, even if the combination was proper, it does not yield the claimed invention. The deficiencies of WO

01/33199, as relating to the claimed invention, are described above. Reichheld et al. does not cure these deficiencies. At best, the combination might yield an assay for inhibitors or stimulators of binding between a transcription factor and histone promoter DNA using the probe structures of WO 01/33199. This is not the claimed invention.

Reconsideration and withdrawal of the rejection under 35 U.S.C. §103(a) in view of WO 01/33199 and Reichheld et al. is respectfully requested.

Tsien et al. (US 2003/0186229) in view of Akhtar et al.

Claims 21-32, 34-36, and 40-45 are rejected under 35 U.S.C. §103(a) as being unpatentable over Tsien et al. (U.S. 2003/0186229) in view of Akhtar et al.

Tsien et al. teaches a chimeric phosphorylation indicator which contains a donor molecule, a phosphorylatable domain, a phosphoaminoacid binding domain, and an acceptor molecule. The phosphorylatable domain can be any molecule that can be phosphorylated by a *specific* kinase or dephosphorylated by a *specific* phosphatase. The domains reported by Tsien et al. are *specific* substrates for the *particular* kinases or phosphatases being assayed. Tsien et al. does not teach that this domain can be a histone polypeptide. The teachings of Akhtar et al. are described above. At a minimum, Akhtar et al. does not teach that histones are phosphorylatable substrates.

A prima facie case of obviousness has not been made. There is no motivation to combine the teachings of Tsien et al. with those of Akhtar et al. because Tsien et al. reports a phosphorylation reporter and Akhtar et al. provides information regarding an acetyltransferase. There is also no motivation to combine the references because Tsien et al. reports a two protein domain reporter construct and Akhtar et al. reports a protein domain (i.e., a chromodomain) that binds to RNA. For at least these reasons, one of ordinary skill would have no reasonable expectation of success that the proteins of Akhtar et al., which are not disclosed as being capable of phosphorylation and which bind to RNA, could be used in the reporters of Tsien et al., which require a phosphorylatable protein domain that binds to another protein domain. Even if the combination was proper (and Applicant maintains it is not), it is unclear what the combination would yield (e.g., the probe of Tsien et al. having MOF as one domain and an RNA molecule as the other domain for use as a phosphorylation reporter). This is not the claimed invention.

The Examiner provides no rationale for the combination of references. In fact, the Examiner cites page 408 of Akhtar et al. and WO 01/33199 in support of the combination. This is an error and Applicant requests clarification.

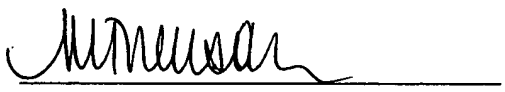
Reconsideration and withdrawal of the rejection under 35 U.S.C. §103(a) in view of Tsien et al. and Akhtar et al. is respectfully requested.

Conclusion

A favorable action is respectfully requested. The Examiner is requested to call the undersigned at the telephone number listed below if this communication does not place the case in condition for allowance.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 23/2825.

Respectfully submitted,



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